High-Throughput Screening and Selection of High Lipid Producers for Biofuels

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OVERVIEW

- Biodiesel production from lipid producing microbial systems is receiving strong interest for their ability to generate sustainable, high-quality fuels. Screening of thousands or even millions of microbial clones is required to find the best lipid producers for further development. Manual selection and further downstream functional screening is time consuming and prone to error.
- *Rhodococcus opacus* was cultured on an agar plate containing Nile Red, a lipophilic fluorescent dye. A QPix 420 automated colony picker was used to select and pick high quality lipid producing *R. opacus* colonies based on morphology and high Nile Red fluorescence intensity.
- The lipid content of the picked colonies was confirmed by BODIPY 505/515 staining, a lipophilic fluorescent dye, and using a SpectraMax M5 Multi-Mode Microplate Reader for fluorescence measurement.
- By picking colonies with high Nile Red fluorescence intensity, it is possible to find desired high lipid colonies in an initial primary screen, limiting downstream functional assays to only the most promising candidates resulting in a significant saving of time and resources.

INTRODUCTION

Environmental and market pressures are driving the demand to discover alternative, renewable energy sources. Researchers are diligently working to engineer lipid producing microbes to develop next generation renewable fuels. To accelerate the discovery of high quality microbial colonies, it is essential not only to incorporate automation, but also functional screening during the colony picking process. Nile Red, a lipophilic fluorescent dye, was used to stain and identify high-lipid producing *Rhodococcus opacus* clones. A QPix 420 automated colony picker with both white light and fluorescence imaging capabilities was used to select and pick colonies based on morphology and Nile Red fluorescence intensity. By identifying and selecting the colonies that have high Nile Red fluorescence, we limit the number of colonies needed for downstream secondary screening or potentially even eliminate the need for additional screening.

EXPERIMENTAL WORKFLOW

Culturing of lipid producing bacteria colonies

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Transfer colonies to agar plates containing Nile Red

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Perform white light and fluorescence imaging, colony screening and picking using QPix 420

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Culture picked colonies and stain with Bodipy 505/515 to confirm lipid levels

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Measure Bodipy 505/515 fluorescence with SpectraMax M5 and image BODIPY 505/515 stained colonies using ImageXpress Micro XLS

METHODS

CULTURING OF BACTERIA

- *Rhodococcus opacus* PD630 (DSMZ culture collection, DSM 44193) was cultured in Trypticase Soy Broth (TSB) medium at 28°C.
- *Escherichia coli* (ATCC, Migula 47076) was cultured in TSB medium at 37°C.
- Liquid cultures of both organisms were grown aerobically in a shaking incubator for 1-3 days.
- *R.opacus* only and a mixture of *R.opacus* and *E.coli* were then cultured on to solid agar plates containing Trypticase Soy Agar (TSA) medium and 0.5 µg/mL of Nile Red (AAT Bioquest, Inc.) for 48-72 hours at 28°C. Nile Red is a lipophilic fluorescent dye that detects lipid production directly in growing bacterial colonies on cultured agar plates.

OBJECTIVE COLONY SCREENING AND PICKING USING WHITE LIGHT AND FLUORESCENCE IMAGING

- White light and fluorescence images of the cultured agar plates were imaged with the QPix 420 Microbial Colony Picker. The QPix 420 FITC filter set (Ex 457nm /Em 536nm) was used to image Nile Red fluorescence.
- Using the QPix software, the colony morphology parameters were adjusted for optimal colony selection. A mean fluorescence intensity (MFI) threshold of greater than 50,000 was set to select colonies showing high Nile Red fluorescence (Figure 2).
- For picking both *R.opacus* and *E.coli* colonies, we used the QPix 420 96-pin picking head with the most optimal picking pins for *E.coli*.
- The picked colonies were transferred to 96-well plates containing liquid TSB medium and incubated overnight at 28°C.

CONFIRMATION OF HIGH LIPID ACCUMULATION COLONY PICKING

- Following overnight incubation, the picked colonies were stained with 0.5 µg/mL of BODIPY 505/515, a lipophilic bright green fluorescent dye, to confirm the lipid levels.
- The SpectraMax® M5 Multi-Mode Microplate Reader and FITC filter set (Ex 485nm / Em 525nm) was used to measure the BODIPY 505/515 fluorescence in the 96-well plates.
- Cell images of the BODIPY 505/515 stained *R.opacus* picked colonies were captured using the ImageXpress® Micro XLS Widefield High Content Screening System and FITC filter set (Ex 482nm / Em 536nm) at 40x magnification.

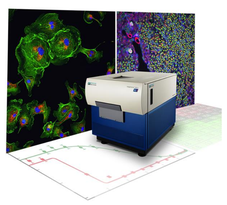
INSTRUMENT OVERVIEW

QPix 420 Automated Colony Picker

- Automated system for screening and selection of colonies
- Images using white light and fluorescence
- Superior picking speed and accuracy
  - Pick up to 30,000 colonies per day
  - > 98% picking efficiency
- Specialized pin designs enable maximal transfer of diverse microorganisms
- Agar level sensor for automated picking pin height adjustment

SpectraMax® M5 Multi-Mode Microplate Reader

- Dual monochromators for assay flexibility
- Patented pathlength correction for better absorbance accuracy
- Superior optics for optimal assay performance
- Comprehensive data analysis and GxP solutions



ImageXpress® Micro XLS Widefield High Content Screening System

- Accelerate screens to > 50,000 wells per day
- Reliable assays with < 5% CV of intensities across a plate
- Wide assay window with 3-log dynamic range
- Complete solution for HCS with integrated software and database packages

FLUORESCENCE PICKING TIME SAVINGS

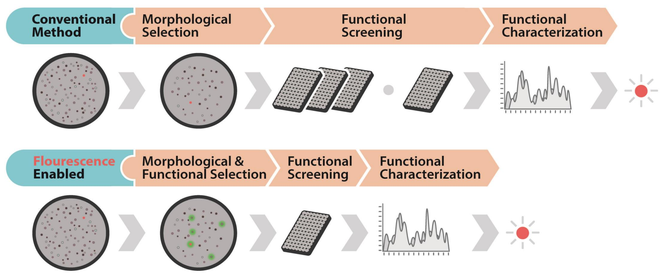


Figure 1. The QPix 400 Systems combine quantitative gain-of-function screening together with highly accurate robotics and integrated software to help shorten development timeline. Fluorescence picking reduces the burden of downstream functional assays by eliminating the majority of low value colonies early.

OBJECTIVE COLONY SCREENING USING THE QPIX 420

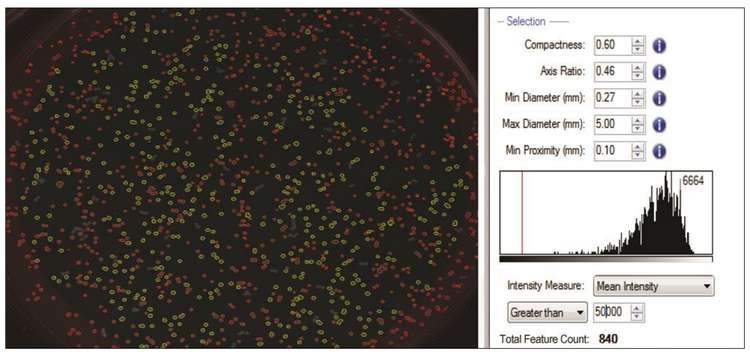


Figure 2. QPix 420 Nile Red fluorescence imaging of *R.opacus* allows for lipid screening of colonies. Along with the morphology selection parameters, a mean fluorescence intensity threshold of 50,000 is set to select high lipid colonies. Colonies that meet these parameters are in yellow and colonies that do not are in red. This allows the early selection of high quality, high lipid producing clones.

QPIX420 NILE RED FLUORESCENCE IMAGING

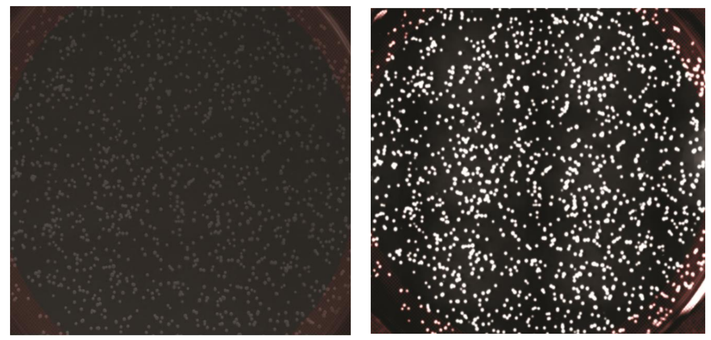


Figure 3. Colonies of *R.opacus* grown in an agar plate containing Nile Red were imaged with a QPix 420 under white light (left) and fluorescence imaging (right). Nile Red staining of high lipid producing colonies results in robust fluorescence.

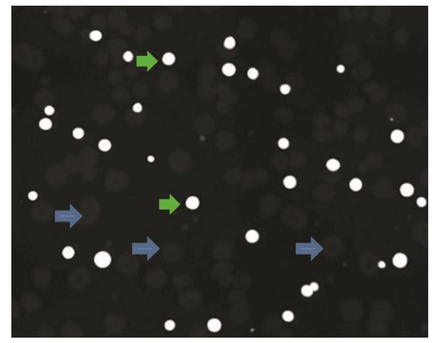


Figure 4. *R.opacus and E.coli* were co-cultured on an agar plate containing Nile Red and fluorescently imaged on QPix 420. Green arrows depict *R.opacus* colonies accumulating high amounts of lipid as observed by bright Nile Red fluorescence; blue arrows indicate *E.coli* colonies that do not exhibit fluorescence. By measuring Nile Red fluorescence, high lipid producing colonies can be easily identified.

BODIPY 505/515 COLONY STAINING RESULTS

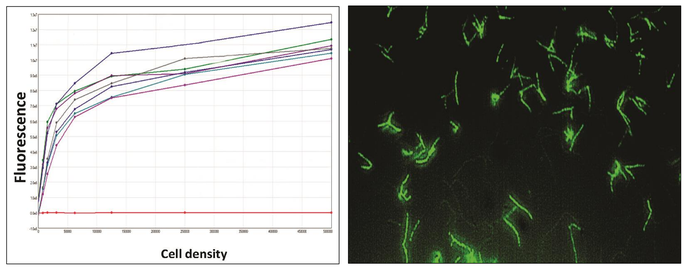


Figure 5. SpectraMax M5 Multi-Mode Microplate Reader fluorescence readings of BODIPY 505/515 stained colonies (left). High lipid producing *R.opacus* colonies exhibited high fluorescence (RFU) values while the *E.coli* colonies exhibited background fluorescence signal as depicted in red. Fluorescent image of high lipid-yielding *R.opacus* stained with BODIPY 505/515 acquired using the ImageXpress Micro XLS Widefield High Content Screening System (right). R.opacus showed high fluorescence as expected. The BODIPY 505/515 staining confirms the lipid levels of the colonies as determined by Nile Red staining.

CONCLUSIONS

- Using an automated microbial colony picker provides a highly efficient and reliable alternative to the conventional manual picking methods. The QPix 420 can accurately picks up to 30,000 colonies per day.
- Colony detection using both white light and fluorescence imaging enables objective identification and selection of colonies based on morphology and a functional marker.
- The QPix 420 and Nile Red staining can be used to detect and pick high lipid accumulating colonies. With the use of the various QPix 420 picking pins and other fluorescent stains, this method can be easily adapted for other microorganisms and markers which will significantly improve the time and cost to identify high value clones.

MOLECULAR DEVICES